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Biological Effects of an Aqueous Extract of Cigarette Smoke Condensate in Rats. I. Effect on Body Weight and Tumor Induction by Benzo(a)pyrene*

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SUMMARY——Graded doses of an aqueous extract of cigarette smoke condensate (AECSC) prepared from a commercial brand of cigarettes were administered in 10% sugar solution as drinking fluid to Sprague-Dawley female rats for 10 weeks starting at 30 days of age. Control animals drank the same volume of sugar solution consumed by the groups given AECSC. The concentration of AECSC influenced fluid intake, which in turn affected body weight. Concentrations higher than 1.0 mg/ml were refused and all animals died of dehydration and starvation. Body weight was significantly depressed at lower concentrations (0.125 to 1.0 mg/ml 10% sugar solution) when compared to animals drinking tap water ad libitum, but only at the 1.0 mg/ml concentration was depression of body weight significantly different from the control group drinking sugar solution alone.

The influence of continuous ingestion of AECSC (0.25 mg/ml 5% sugar solution) as drinking fluid on benzo(a)pyrene (B(a)P) induced sarcoma was studied in 8 groups of 20 female Sprague-Dawley rats. Comparisons were made with similar groups given 5% sugar solution to drink. Doses of B(a)P ranging from 3 to 400 μg dissolved in 0.1 ml sesame oil were administered by subcutaneous injection on alternate days for 30 doses. All animals injected with 12.5, 25, 50, 100, 200 or 400 μg B(a)P developed fibrosarcoma at the site of injection in 80 to 126 days. All rats injected with 3 μg B(a)P per dose (total dose 90 μg) and given AECSC to drink developed tumors in 235 ± 15 da (M ± S.E.) but only 9 of 19 given sugar solution as drinking fluid had sarcoma during an observation period of 1 yr. These observations suggest that AECSC contains one or more components which function as a co-carcinogen. However, AECSC did not accelerate the appearance of spontaneous tumors.

Innumerable studies have been reported on the carcinogenic potency of tobacco tar, whole smoke condensate and the various fractions of tobacco smoke condensate. That whole tobacco smoke condensate or its neutral fraction is weakly carcinogenic when applied to the mouse back is beyond question, but in no case has all the carcinogenicity of whole tobacco smoke condensate been accounted for satisfactorily by the assay of its various fractions. Roe, et al. (1) and Boutwell and Bosch (2) have demonstrated that phenols of tobacco smoke condensate are tumor-promoting. Dickens and Jones (3) and Van Duuren, et al. (4) have shown that certain lactones are carcinogenic, but these substances have not been identified in tobacco smoke condensate. Nitrosamines have been suspected but again there is no firm evidence that these compounds are present. Whitehead and Rothwell (5) assayed an aqueous fraction of cigarette smoke condensate and observed a low incidence of papilloma and carcinoma in mice, but concluded that the observations were not significant. Other than a report by Bock (6) that aqueous extracts of unburned tobacco possessed tumor-promoting effects, data on this fraction has not been published. The experiments to be described indicate that the aqueous fraction contains one or more components which function as a co-carcinogen.

MATERIALS AND METHODS

Preparation of materials—Aqueous extract of cigarette smoke condensate (AECSC) was prepared from a commercial brand of cigarettes by the Smoke Preparations Laboratory, University of Kentucky, under the direction of Dr. John Green. The method of preparation is shown in text-figure 1. A fresh batch of condensate was prepared and used each week. When not in use it was stored in a light-protected polyethylene bottle at 10^{o}. Benzo(a)pyrene (Eastman Distillation Industries) was purified in hexane-benzene 9:1 v/v on a florisil column, dried under reduced pressure, and recrystallized in benzene. The desired concentrations were dissolved in sesame oil and stored in sealed amber vials at room temperature.

Animal experiments—Weanling female rats (purchased from Sprague-Dawley Farms, Madison, Wis.) were acclimatized for 8 days in a constant-temperature animal room (74°F) with an alternating light-dark cycle of 12 hours. They were housed in stainless steel cages, 5 rats per cage, and given Purina rat chow and tap water ad libitum. At 30 days of age they were divided into 2 experimental groups; one group was given AECSC in 5% or 10% sugar solution as drinking fluid and the other group was given the same volume of sugar consumed by the group drinking AECSC. Two experiments to determine the tolerance of rats for AECSC were conducted. In these experiments AECSC was administered in 10% sugar solution. In the two experiments to determine the influence of AECSC on benzo(a)pyrene-induced sarcomata, AECSC 0.25 mg/ml of 5% sugar solution was given as drinking fluid. In one experiment benzo(a)-pyrene, B(a)P, 400, 200, or 100 μg, and in the other 50,.25, 12.5 or 3 μg dissolved in 0.1 ml sesame oil was injected under the skin of the back on alternate days for 30 doses. The sites of injection were changed each time so that there was an orderly progression from the nape of neck to mid-back to lower back. Before each injection all oil deposits were broken up by gentle massage. All animals were weighed each week and killed 33 ± 3 days after the detection of the first palpable tumor. On the day prior to sacrifice, hematocrit and total and differential leucocyte counts were performed in duplicate from blood obtained by lancing the tail. Smears of femoral marrow from representative animals were obtained at autopsy. Tumors and organs were weighed and stored in 10% neutral formalin until processed by standard methods for microscopic examination. All tumor-negative animals were observed for one year with the exception of sesame oil-injected rats serving as controls for the groups given 400, 200 or 100 μg doses of B(a)P.

To determine whether AECSC influenced the development of benzo(a)pyrene-induced mammary cancer two groups of rats that had been drinking AECSC (0.25 mg/ml 5% sugar solution) and two groups given the same volume of 5% sugar solution alone for three weeks were fed B(a)P as a single dose, 100 mg in 5 ml sesame oil, or 20 mg in 1 ml sesame oil for 5 consecutive weeks. Comparable groups were fed an identical volume of sesame oil. These animals were killed 2 months after the appearance of the first palpable mammary tumor. All tumor-negative animals were observed for one year.

RESULTS

Influence of AECSC on body weight— Text-figure 2 shows the changes in body weight when various doses of AECSC were administered. In all cases the amount of fluid consumed was proportional to the concentration of AECSC. The animals refused to drink concentrations higher than 1 mg/ml and died of dehydration and starvation. Only at the 1 mg/ml concentration was there a definite effect on body weight which could not be attributed to inadequate fluid intake per se. Organ weights (table 1) reflected changes in body weight. At the lowest concentration, 0.125 mg/ml, body weight was slightly but not significantly lower than that of comparable groups drinking tap water ad libitum. Adolph (7) and Collier and Levitsky (8) have shown that rats restrict food intake in proportion to fluid intake, hence weight loss is due to both factors. The component(s) of AECSC which are responsible for the voluntary restriction of fluid intake have not been identified, but preliminary (unpublished) data indicate that it is not nicotine. Both Passey (9) and Haag, et al. (10) have reported that rats exposed to atmospheric smoke gained less weight than non-exposed animals.

Table 1—Mean body and organ weights of Sprague-Dawley female rats, age 100 da, given an aqueous extract of cigarette smoke condensate (AECSC) in 10% sugar sol. as drinking fluid for 70 da. Control rats drank the same quantity of fluid (10% sugar sol.) consumed by the experimental group.

	AECSC 1 mg/ml	Control	AECSC 0.5 mg/ml	Control	AECSC 0.25 mg/ml	Control	AECSC 0.125 mg/ml	Control
Body weight (gm)	141*	165	176	186	201	205	225	215
Liver	4.42	4.95	6.68	7.10	7.33	7.06	7.72	6.78
Kidneys†	1.15*	1.34	1.53	1.55	1.70	1.78	1.58	1.58
Brain	1.49	1.54	1.49	1.51	1.57	1.56	1.60	1.60
Lungs†	0.70	0.86	1.02	1.12	1.48	1.35	1.32	1.14
Heart	0.45	0.51	0.57	0.63	0.65	0.68	0.78	0.76
Spleen	0.31*	0.43	0.47	0.47	0.50	0.48	0.58*	0.46
Thymus	0.16*	0.27	0.23	0.25	0.27	0.31	0.27	0.28
Uterus	0.18*	0.27	0.31	0.38	0.45	0.45	0.44	0.45
Ovaries†	0.055*	0.078	0.085	0.089	0.094	0.101	0.108	0.099
Adrenals†	0.040*	0.049	0.054	0.053	0.059	0.057	0.065	0.057
Pituitary	0.005	0.005	0.010	0.010	0.010	0.011	0.013	0.012
Hct (%)	50	48					49	49
Leucocytes/cu mm	7280	7050					14300*	7540

* Difference between experimental and control significant, P = >0.05
† Paired organs

Effect of AECSC on tumor induction. Subcutaneous injection of B(a)P—The incidence of tumors and the mean (± S.E.) tumor-induction time is shown in table 2. All tumors present at the site of injection were fibrosarcomas. In the first of these experiments mean tumor induction time (the interval in days from initial injection of B(a)P until the detection of tumor by palpation) was shorter for the groups given AECSC to drink. The mean (± S.E.) tumor induction time was 79 ± 3.6, 83.4 ± 2.7, and 80.2 ± 2.8 days for the groups given 400, 200, or 100-microgram doses of B(a)P; for comparable groups given sugar solution to drink it was 95 ± 3.8, 98 ± 3.8, and 96 ± 4 days. It should be emphasized that the above values are probably higher than the true tumor induction time because the local inflammatory reaction, induration and thickening of the subcutis due to B(a)P precluded detection of small tumors. Hence, tumor-induction time was estimated from the day that a definite increase in the size of the suspected tumor occurred. Mean tumor weight was less in 2 of the 3 groups given AECSC but there was no difference in the mean number of tumors per rat in the 6 groups injected with B(a)P. None of the rats injected with sesame oil had detectable tumors when they were killed at 22 weeks, age 185 days.

In the second experiment tumor-induction time could be assessed with greater accuracy since there was less local reaction to injection of B(a)P. All rats injected with 50, 25, and 12.5 μg developed sarcomas at the site of injection. With the exception of the 2 groups injected with the 25-μg dose, tumor-induction time for comparable groups (AECSC versus sugar solution) was in agreement but mean tumor weight for the groups drinking AECSC was somewhat higher. The difference in tumor-induction time between the 3 and the 12.5, and between the 12.5 and the 25-μg dose was highly significant ($P \leq .01$) for both groups; the difference between the 50 and 25-μg dose was significant for the groups drinking sugar solution. The effect of AECSC is most clearly manifest in the groups injected with the 3-μg dose of B(a)P. This dose is equivalent to the B(a)P content of 50 cigarettes (11,12). All rats given AECSC to drink had cancer with a mean ($\pm$ S.E.) tumor induction time of 235 $\pm$ 15 days. Nine of 19 animals given sugar solution to drink had tumors in 272 $\pm$ 19 days and 10 were free of cancer when they were sacrificed at 14 months of age. (One rat in the group given AECSC was killed at 212 days when she became moribund as a consequence of uterine cancer, and one rat in the group given sugar solution was sacrificed at 245 days with an eroding fibroadenoma of the breast. Neither of these animals had tumors at the site of injection and are excluded from the tabular data.) The difference in mean tumor induction time between these two groups is not significant but the difference in tumor incidence (100% versus 45%) provides the most convincing evidence that one or more compounds in the aqueous fraction of cigarette smoke condensate accelerate(s) the induction of cancer by B(a)P.

Table 2. Influence of aqueous extract of cigarette smoke condensate (AECSC) on induction of sarcoma by benzo(a)pyrene, B(a)P, in Sprague-Dawley female rats.

Dose of B(a)P[1] Single (μg)	Total (mg)	Drinking solution	Rats with sarcoma	Tumor induction time[2] (da) Mean ± S.E.	Tumors per rat (mean)	Total tumor weight[3] (gm) Mean ± S.E.
Experiment 1						
100	3.0	AECSC[4]	20/20	80.2 ± 2.8	2.9	30.9 ± 6.8
200	6.0	AECSC	19/19	83.4 ± 2.7	2.7	12.8 ± 2.9
400	12.0	AECSC	20/20	79.7 ± 3.6	2.6	11.0 ± 2.2
Sesame oil		AECSC	0/20			
100	3.0	5% sugar	18/18	96.0 ± 4.0	2.8	28.8 ± 6.7
200	6.0	5% sugar	19/19	98.0 ± 3.8	3.1	29.7 ± 9.1
400	12.0	5% sugar	18/18	95.0 ± 3.8	3.1	24.8 ± 8.8
Sesame oil		5% sugar	0/20			
Experiment 2						
3	0.09	AECSC	19/19	235.0 ± 15.0	1.1	8.4 ± 2.8
12.5	0.375	AECSC	20/20	125.0 ± 6.5	1.7	24.7 ± 6.9
25	0.75	AECSC	20/20	98.0 ± 2.6	2.0	25.2 ± 6.7
50	1.500	AECSC	20/20	93.0 ± 4.3	1.9	20.1 ± 5.3
Sesame oil		AECSC	0/20			
3	0.09	5% sugar	9/19	272.0 ± 19.0	1.1	4.8 ± 1.7
12.5	0.375	5% sugar	20/20	126.0 ± 6.5	2.0	17.3 ± 4.4
25	0.75	5% sugar	20/20	111.0 ± 3.7	2.0	16.5 ± 3.6
50	1.500	5% sugar	20/20	95.0 ± 5.0	2.0	11.5 ± 2.2
Sesame oil		5% sugar	0/20			

[1] B(a)P, dissolved in 0.1 ml sesame oil, was administered by subcutaneous injection on alternate days for 30 doses, starting at 30 days of age.

[2] The number of days from initial injection of B(a)P until detection of tumor by palpation.

[3] On necropsy 30-36 days after detection of tumor.

[4] AECSC was dissolved in 5% sugar solution; control groups drank a comparable volume of 5% sugar solution.

Tumor free animals in Experiment 1 were observed 5 months and those in Experiment 2 for one year.

The results of these experiments indicate there is no consistent dose-response relationship at concentrations higher than 50 μg B(a)P if tumor-induction time is considered to be the response and even at the lower doses there must be a four-fold difference in dose (50 μg vs. 12.5 μg) to bring out highly significant differences with the multiple injection technic. If incidence of cancer is a measure of the response, the true dose-response curve lies below the 12.5 μg dose.

Van Duuren, et al. (13) tested whole tobacco extract (WTE) or whole cigarette tar (WCT) on the shaved mouse back using DMBA or B(a)P as an initiator. According to their data, 500 μg B(a)P was a weak initiator and carcinogenesis was not appreciably enhanced by the promoters, WTE or WCT. Croton oil alone seemed to be as carcinogenic as the above combinations. The high incidence of tumors observed in rats given multiple injections of low doses of B(a)P suggest that the mouse-back assay system has a low order of sensitivity.

In their studies with mice Shimkin and Andervont (14) concluded that the total dose of carcinogen was more important than frequency of injections. This statement is probably correct for high doses of B(a)P but does not appear to be applicable when relatively low doses are employed. To test this hypothesis 400 μg B(a)P in 0.1 ml sesame oil was given to female rats, age 30 days, by subcutaneous injection once only. Fifteen of 18 rats developed sarcoma at the site of injection in 204 ± 21 days (M ± S.E.) and 3 were free of tumors when sacrificed at 375 days, whereas all animals given a total dose of 375 μg (12.5 μg per dose) developed tumors with a mean (± S.E.) tumor induction time of 126 ± 6.5 days. In another experiment 5 female rats, age 35 days, were given a subcutaneous injection of 2 mg B(a)P on alternate days for 6 doses. At autopsy, 110 days later, there was no evidence of tumor. These observations suggest that the total dose of carcinogen may not be as critical as the duration of the exposure time to the compound. On the other hand, the larger dose, 2 mg x 6, could have destroyed all potential cancer cells, since it induced marked hemorrhagic necrosis of the subcutis at the site of injection.

Since each laboratory which has reported observations on rats after injection of B(a)P used different experimental conditions, it is difficult to evaluate the results presented in this report vis a vis those from other laboratories. Rözynek (15) reported that 3 of 9 rats injected with 1 mg B(a)P had tumors at 91 days and 8 of 9 at 174 days, but made no statement regarding the size or morphology of the tumors induced. Hüter (16) employed a single dose of 10 mg in 0.5 ml olive oil and observed sarcomata in 10 of 15 animals (67%) at 12-16 weeks. Neither the age of the rats nor the mean latent period were given. Zamurovitch (17) used a single 5-mg dose dissolved in tournesol. Thirteen of 15 rats developed tumors between 120 and 280 days with a mean tumor induction time of 150 days. Dunning, et al. (18) injected 2 mg B(a)P dissolved in paraffin at separate sites using rats of different strains. Tumor induction time was 118 $\pm$ 2.0 days for the August strain, and 177 $\pm$ 11 days for the Silver Grey strain. Huggins, et al. (19) observed that the intramuscular injection of 2.5 mg in 0.5 ml sesame oil in the hind leg of 8 Long-Evans strain male rats, age 24 days, induced sarcoma at the site of injection in 116 $\pm$ 8 days. Druckrey and Schildbach (20) injected B-D strain rats with 30, 10, or 3-µg doses of B(a)P at weekly intervals for many months. Twenty-five percent of their animals developed sarcomata in 200, 460, and 700 days when 30, 10 or 3-µg doses were employed. The total dose of B(a)P administered to attain a D_{25} level was 300, 660, and 800 µg respectively. Differences in rat strain, age, frequency of administration of the compound, and the solvent employed by other investigators preclude evaluation of our observations on a comparative basis. We can only conclude that the Sprague-Dawley rat is extremely sensitive to low doses of this carcinogen when it is administered repeatedly by the subcutaneous route for a limited period of time. Additional modifications of the dose-regimen may further enhance the carcinogenic response.

The mean (± S.E.) values for organ weights recorded at autopsy for the animals used in experiment 1 are shown in the appendix, tables app-1 (AECSC solution) and app-2 (sugar solution) and those for the animals in experiment 2 in tables app-3 (AECSC) and app-4 (sugar solution). Organs that are significantly higher than the mean control values are summarized in table 3. Net body weight (autopsy weight minus total tumor weight) for all groups injected with doses of B(a)P higher than 3 μg was in agreement but gross body weight in the B(a)P-treated animals in the first experiment was higher than their controls. Difference in body weight may account for their heavier hearts, lungs, and adrenals since no pathological changes were observed in these tissues. Liver was significantly heavier in all groups injected with B(a)P than their sesame-oil treated controls. With the exception of the group injected with 3 μg and given sugar solution to drink, spleen weight was significantly greater. This group included 10 rats without cancer. In the control groups observed for 1 yr. mean liver and spleen weight was greater in the group given AECSC but the differences were not significant.

Table 3.-- Organ weight significantly heavier ($P = > .02$) than control groups injected with sesame oil.

	Lungs	Heart	Liver	Spleen	Adrenals
Experiment 1					
BP 400 µg + AECSC	+	+	+	+	+
BP 400 µg + Sugar	+	+	+	+	+
BP 200 µg + AECSC	+	+	+	+	+
BP 200 µg + Sugar	+	-	+	+	-
BP 100 µg + AECSC	+	+	+	+	-
BP 100 µg + Sugar	+	-	+	+	-
Experiment 2					
BP 50 µg + AECSC	-	-	+	+	+
BP 50 µg + Sugar	-	-	+	+	+
BP 25 µg + AECSC	-	-	+	+	-
BP 25 µg + Sugar	-	-	+	+	-
BP 12.5 µg + AECSC	-	-	+	+	-
BP 12.5 µg + Sugar	-	-	+	+	+
BP 3 µg + AECSC	-	-	+	+	-
BP 3 µg + Sugar	-	-	+	-	-

The hemopoietic indices are shown in table 4. When compared with their respective control groups (injected with sesame oil) mean leucocyte count is significantly higher in all groups given B(a)P but there is no correlation with dose. When the data are re-arranged according to tumor weight, (tables 5 and 6) the relationship between leucocytosis, granulocytosis, hepatosplenomegaly, and body weight are more clearly delineated. For the most part all tumors were solid, non-necrotic fibrosarcomas with an abundant capillary network, the large vessels being confined to the periphery of the tumors. Observations on animals bearing hemorrhagic or grossly necrotic tumors are excluded from these calculations. The observations for the two experiments are in general agreement. Thus, tumors weighing more than 5 gm induce leucocytosis which increases with tumor weight, a phenomenon described by Parsons (21) for mice injected with sodium 1:2:5:6-dibenzanthracene-9;10-endo-alpha,beta succinate and by Dunning and Reich (22) for rats injected with benz(a)pyrene or 3-methylcholanthrene. Hepatosplenomegaly was present in animals bearing tumors weighing more than 15 gm. Spleens from these animals showed the greatest deviation from normal (figs. 1 and 2), being grossly enlarged, hypercellular and packed with giant multinucleated cells which undergo digestion with taka-diastase. It is our opinion that these cells are megakaryocytes. The first organ to become significantly heavier is liver, but other than a large number of Kupfer cells, routine sections stained with hematoxylin and eosin showed no distinct morphological changes. Bone marrows from animals with the highest leucocyte counts (40000 to 60000 per cu mm) were hypercellular, but the cells were normal in appearance. The most distinguishing feature of the peripheral blood smears was an increase in the number of large binucleated lymphocytoid cells in the groups given AECSC to drink (fig. 3). In this group control animals observed for one year had a slightly but significantly lower mean leucocyte count ($P = .02$) and hematocrit ($P = .05$) than the control group given sugar solution to drink. No explanation for these findings is apparent at this time.

Table 4. Mean (± S.E.) leucocyte count and hematocrit in tail blood from Sprague-Dawley female rats one day preceding autopsy

No. Rats	Treatment[1]	Hematocrit (%)	Leucocytes (cu mm)	Granulocytes (%) Neut.[2]	Granulocytes (%) Eos.[3]	Lymphocytes + Monocytes (%) Small	Lymphocytes + Monocytes (%) Large
Experiment 1							
20	BP 400 µg + AECSC	45.1 ± 0.91	15532 ± 400	22 ± 3.1	0.9 ± 0.12	44 ± 2.5	33 ± 2.6*
20	BP 400 µg + Sugar	43.9 ± 1.01	16236 ± 659	26 ± 4.0	0.9 ± 0.13	61 ± 3.2	12 ± 3.4
19	BP 200 µg + AECSC	44.2 ± 0.72	14108 ± 906	26 ± 2.2	1.0 ± 0.10	50 ± 2.4	23 ± 2.8*
19	BP 200 µg + Sugar	44.7 ± 0.98	19032 ± 3087	30 ± 3.4	1.3 ± 0.11	56 ± 2.8	12 ± 2.0
20	BP 100 µg + AECSC	43.2 ± 1.21	19781 ± 2091	34 ± 2.6	0.4 ± 0.12	35 ± 2.6	31 ± 2.4*
19	BP 100 µg + Sugar	42.3 ± 0.37	17065 ± 1931	32 ± 3.3	0.9 ± 0.12	36 ± 2.1	22 ± 2.6
15	Sesame oil + AECSC	46.9 ± 0.80	7130 ± 398	11 ± 1.2	0.9 ± 0.05	44 ± 3.1	33 ± 2.0*
20	Sesame oil + Sugar	47.9 ± 0.45	7880 ± 615	13 ± 2.1	0.4 ± 0.03	67 ± 3.2	18 ± 1.8
Experiment 2							
20	BP 50 µg + AECSC	44.3 ± 0.4	22377 ± 2353*	25 ± 2.7*	1.0 ± 0.11	56 ± 3.2	18 ± 1.6
20	BP 50 µg + Sugar	45.0 ± 0.9	14064 ± 1383	15 ± 2.7	1.0 ± 0.16	68 ± 3.1	16 ± 1.3
20	BP 25 µg + AECSC	42.9 ± 0.5	22028 ± 2891	26 ± 3.6	1.0 ± 0.16	51 ± 3.4	22 ± 1.9
20	BP 25 µg + Sugar	43.7 ± 0.8	19336 ± 1784	30 ± 2.6	1.0 ± 0.12	51 ± 3.6	18 ± 1.9
19	BP 12.5 µg + AECSC	42.8 ± 0.7*	16020 ± 1440	25 ± 2.8	1.0 ± 0.11	51 ± 3.2	23 ± 2.0
20	BP 12.5 µg + Sugar	44.8 ± 0.6	15886 ± 1393	26 ± 2.7	1.1 ± 0.14	55 ± 3.3	18 ± 1.5
20	BP 3 µg + AECSC	46.1 ± 0.5*	15606 ± 3037	23 ± 2.7	1.6 ± 0.20	51 ± 3.7*	24 ± 2.7*
20	BP 3 µg + Sugar	44.8 ± 0.5	12072 ± 879	22 ± 2.6	1.6 ± 0.30	64 ± 2.7	12 ± 1.5
19	Sesame oil + AECSC	45.3 ± 0.5*	9066 ± 357*	24 ± 2.7	3.8 ± 0.40*	57 ± 3.7	15 ± 2.3
19	Sesame oil + Sugar	46.2 ± 0.2	10768 ± 576	19 ± 3.5	1.8 ± 0.20	65 ± 3.9	14 ± 1.7

[1] Subcutaneous injection of benzo(a)pyrene (BP) on alternate days for 30 doses, age 30 to 90 da, and aqueous extract of cigarette smoke condensate (AECSC) 0.25 mg/ml of 5% sugar sol. or a comparable volume of 5% sugar sol. as drinking fluid from 30 da. of age until autopsy. Tumor-bearing animals were killed 33 ± 3 da. after detection of the first palpable tumor. Control animals injected with sesame oil were observed for 22 weeks in Experiment 1 and 55 weeks in Experiment 2.

[2] Neutrophiles

[3] Eosinophiles

* Significantly different from comparable group drinking 5% sugar sol., P =>.02

Table 5. Relationship of mean (± S.E.) tumor weight to body, spleen, and liver weights and leucocytosis. (Tumors were induced by the subcutaneous injection of 100, 200 or 400 μg benzo(a)pyrene (BP) on alternate days for 30 doses. Tumor-bearing animals were killed 33 ± 3 days after detection of the first tumor by palpation. Tumor-negative animals were observed for 22 wk.

Total tumor wt/rat	<5 gm	5-15 gm	15-30 gm	>30 gm	None
No. of rats					
AECSC	17	18	12	10	15
Sugar	17	13	7	16	20
Autopsy weight					
AECSC	237. ± 4.1	244. ± 4.1	249. ± 4.1	276. ± 8.1	229. ± 3.3
Sugar	229. ± 5.0	235. ± 5.2	250. ± 5.2	284. ± 8.8	233. ± 2.9
Tumor weight					
AECSC	2.9 ± 1.2	9.53 ± 0.82	20.2 ± 1.18	57.7 ± 7.6	---
Sugar	2.4 ± 0.3	10.32 ± 0.92	20.5 ± 1.96	62.2 ± 9.7	---
Net body weight†					
AECSC	233.9	235.5	226.8	218.3	229.0
Sugar	226.6	224.7	229.8	221.8	233.0
Liver					
AECSC	8.82 ± 0.17*	8.81 ± 0.22	10.19 ± 0.38*	10.95 ± 0.53	7.04 ± 0.18
Sugar	7.66 ± 0.25	8.40 ± 0.28	8.41 ± 0.23	11.87 ± 0.64	7.50 ± 0.23
Spleen					
AECSC	0.550 ± 0.017	0.684 ± 0.042	0.911 ± 0.081*	0.994 ± 0.129	0.490 ± 0.015
Sugar	0.545 ± 0.010	0.697 ± 0.050	0.586 ± 0.031	1.291 ± 0.128	0.488 ± 0.017
Leucocytes (cu mm)					
AECSC	14268 ± 882	15345 ± 1263	17975 ± 1523*	23550 ± 3097	7130 ± 398
Sugar	12100 ± 1143	14537 ± 1141	13104 ± 1470	27118 ± 3489	7880 ± 615
Neutrophiles (%)					
AECSC	22.0 ± 2.0	23.0 ± 2.4	31.0 ± 2.8	41.0 ± 3.4	11.0 ± 1.2
Sugar	18.0 ± 1.9	23.0 ± 3.1	32.0 ± 4.0	42.0 ± 3.4	13.0 ± 2.1
Hematocrit (%)					
AECSC	44.7 ± 0.4*	44.9 ± 0.7	43.8 ± 1.5	41.9 ± 1.5	46.9 ± 0.8
Sugar	46.5 ± 0.5	45.1 ± 1.0	45.6 ± 1.5	38.9 ± 1.9	47.9 ± 0.5
Large lymphocytes and monocytes (%)					
AECSC	20.0 ± 3.8	24.0 ± 3.2*	31.0 ± 4.7*	24.0 ± 4.3*	33.0 ± 2.0*
Sugar	18.0 ± 2.9	7.0 ± 2.6	12.0 ± 3.6	14.0 ± 2.1	18.0 ± 1.8

* Significantly different from comparable group drinking 5% sugar solution
† Net body weight = body weight at autopsy minus tumor weight

Table 6. Relationship of mean (± S.E.) tumor weight to body, spleen, and liver weights and leucocytes. (Tumors were induced by the subcutaneous injection of benzo(a)pyrene (BP), 3, 12.5, 25, or 50 µg, on alternate days for 30 doses. Tumor-bearing animals were killed 33 ± 3 days after detection of the first tumor by palpation. Tumor-negative animals were observed for 1 yr.

Total tumor wt/rat	<5 gm	5-15 gm	15-30 gm	>30 gm	None
No. of rats					
AECSC	30	18	10	20	20
Sugar	27	16‡	12	9	20
Autopsy weight					
AECSC	243. ± 7.4	247. ± 2.8	271. ± 4.0	281. ± 5.5	255. ± 4.0
Sugar	239. ± 3.1	250. ± 3.3	255. ± 4.0	272. ± 5.6	271. ± 7.0
Tumor weight					
AECSC	2.2 ± 0.27	7.9 ± 0.67	21.2 ± 1.40	56.5 ± 5.62	---
Sugar	2.5 ± 0.21	10.7 ± 0.63	19.4 ± 0.84	48.6 ± 5.54	---
Net body weight†					
AECSC	241.	239.	250.	225.	---
Sugar	237.	240.	236.	224.	---
Liver					
AECSC	7.83 ± 0.14	8.03 ± 0.20	8.89 ± 0.37	10.27 ± 0.34	7.54 ± 0.22
Sugar	7.95 ± 0.16	8.36 ± 0.27	9.46 ± 0.27	10.77 ± 0.47	7.19 ± 0.22
Spleen					
AECSC	0.50 ± 0.05	0.56 ± 0.04	0.67 ± 0.03	1.04 ± 0.09	0.53 ± 0.01
Sugar	0.53 ± 0.01	0.58 ± 0.02	0.70 ± 0.05	0.92 ± 0.11	0.50 ± 0.01
Leucocytes (cu mm)					
AECSC	10924 ± 384	13189 ± 1207	23959 ± 2480	36585 ± 2755	9066 ± 357*
Sugar	9888 ± 497	13034 ± 619	19045 ± 1186	30600 ± 3396	10768 ± 576
Neutrophiles (%)					
AECSC	15.8 ± 1.44	21.5 ± 4.74	33.3 ± 3.59	37.7 ± 2.56	24.2 ± 2.72
Sugar	12.7 ± 1.24	24.1 ± 2.55	31.2 ± 3.21	34.7 ± 3.15	19.4 ± 3.50
Hematocrit (%)					
AECSC	46.6 ± 0.33	42.9 ± 0.47	43.1 ± 0.70	42.5 ± 0.61	45.3 ± 0.51*
Sugar	46.6 ± 0.25	44.9 ± 0.60	44.1 ± 0.54	40.2 ± 1.29	46.2 ± 0.23

‡ 5 animals with necrotic or hemorrhagic tumors are excluded.
† Net body weight = body weight at autopsy minus tumor weight.
* Significantly different from comparable group drinking 5% sugar sol., P =>.05.

The occurrence of leucocytosis, granulocytosis, and hepato-splenomegaly is interpreted as myeloid hyperplasia and is a common occurrence in experimental animals bearing transplanted tumors (23,24). Lappat and Cawein (25) reviewed the literature on this subject and concluded on the basis of their experiments that hepato-splenomegaly was due to extramedullary hemopoiesis. Delmonte and Liebelt (26) have demonstrated that granulopoietin is present in certain tumors and other investigators have observed an increase in serum enzymes (27,28) and glycoproteins (29,30) in tumor-bearing rats. The experimental evidence indicates that the tumor stimulates the reticulo-endothelial system via several mechanisms. We have not found an earlier report of myeloid hyperplasia in rats bearing primary tumors induced by benzo(a)pyrene.

Tumors at other sites—In the first experiment two rats in each group of 60 (AECSC versus sugar solution) injected with B(a)P had mammary fibroadenoma, 2 injected with 400 μg and given 5% sugar solution as drinking fluid had renal carcinoma (fig. 4) and one injected with 200 μg and given AECSC had carcinoma of the bladder. No tumors were observed in the two control groups injected with sesame oil that were killed at 22 weeks. In the second experiment, two of 80 rats in each group injected with B(a)P had mammary fibroadenoma, and one rat given the 3-μg dose and AECSC to drink had a large tumor of the uterus which on microscopic examination proved to be a myosarcoma. None of the 20 control rats given AECSC to drink and observed for one year had tumors, but 6 of 20 given sugar solution had breast tumors, one with adenocarcinoma and five with fibroadenoma. The incidence of these tumors falls within the range of spontaneous tumors observed by other investigators (31).

Tumors induced by feeding benzo(a)pyrene—Rats fed 100 mg B(a)P either as a single dose or as 20-mg doses once each week for five doses had relatively few mammary cancers. After a single feeding at age 51 days, mammary cancer was observed in 4 and mammary fibroadenoma in 4 of 20 rats given AECSC as drinking fluid; 14 rats were free of macroscopically detectable tumors when sacrificed at 14 months of age. In the group given sugar solution 4 rats had mammary adenocarcinoma, 10 had mammary fibroadenoma, and 6 were free of tumors. When 20 mg of B(a)P were fed once each week for five doses, 4 of 20 rats given AECSC had mammary cancer, 2 had mammary fibroadenoma, and 14 were tumor-negative; 6 of 20 rats given sugar solution had mammary cancer, 3 had mammary fibroadenoma, one had renal carcinoma, one had skin cancer, and one had fibrosarcoma of the uterus; 11 of 20 rats were free of cancer and 9 were free of tumor. These observations are summarized in table 7. In general rats given 5% sugar solution to drink had a higher incidence of mammary fibroadenoma whereas those given AECSC in 5% sugar solution had a higher incidence of uterine pathology. Uterine pathology is attributed to degenerated uterine polyps which were manifested by vaginal bleeding. Resorption of blood and subsequent infection could account for pyometria. Microscopic examination of ovaries from these animals revealed no pathological changes.

Although Huggins and Yang (32) observed mammary cancer in 8 of 9 female Sprague-Dawley rats fed 100 mg B(a)P in sesame oil, Pataki and Huggins (33) later reported an incidence of 30% when a larger series of animals was employed. Our observations are in agreement with those reported by Pataki and Huggins. AECSC had no influence on the incidence or tumor-induction time of breast cancer induced by B(a)P.

Table 7. Incidence of tumors and other pathology in Sprague-Dawley female rats 13 months after feeding benzo(a)pyrene (BP) or sesame oil by gastric tube at age 51 days. In one group a total dose of 100 mg carcinogen was administered as a single feeding; in the other group 20 mg were fed at weekly intervals for 5 doses. The data for the two groups of rats given sesame oil are combined.

Drinking Fluid	AECSC[1] in 5% Sugar Sol.			5% Sugar Sol.		
Treatment	BP 100 mg x 1 dose	BP 20 mg x 5 doses	Sesame Oil	BP 100 mg x 1 dose	BP 20 mg x 5 doses	Sesame Oil
Tumors						
Mammary adenoca.	4/20	4/20	2/40	4/20	6/20	1/40
Mammary fibroadenoma	4/20	2/20	4/40	10/20	3/20	4/40
Renal adenoca.	0/20	0/20	1/40	0/20	1/20	0/40
Fibrosarcoma of uterus	0/20	0/20	0/40	0/20	1/20	0/40
Ear duct tumor	1/20	0/20	0/40	0/20	0/20	0/40
Skin cancer	0/20	0/20	0/40	0/20	1/20	0/40
Rats without tumors	14/20	14/20	33/40	6/20	9/20	35/40
Cataract	0/20	4/20	3/40	2/20	1/20	1/40
Uterine pathology:						
Polyps	0/20	1/20	1/40	2/20	4/20	1/40
Hematometria	5/20	4/20	3/40	1/20	2/20	0/40
Pyometria	3/20	2/20	7/40	1/20	2/20	2/40
Galactocele	3/20	0/20	2/40	0/20	0/20	0/40

[1] Aqueous extract of cigarette smoke condensate, 0.25 mg/ml 5% sugar solution

Mean body and organ weights for the groups given 100 mg B(a)P as a single feeding and those given 20 mg B(a)P each week for five doses (total dose 100 μg) are shown in app. 5 and app. 6 respectively. Other than a slightly higher mean liver weight in the groups fed B(a)P, the data for control and experimental animals are in excellent agreement.

Of the 40 control animals given AECSC 2 rats had mammary adenocarcinoma, 4 had mammary fibroadenoma and one had adenocarcinoma of the kidney; in the two groups given sugar solution 4 of 40 had mammary fibroadenoma and one had mammary adenocarcinoma. When these figures are combined with the two control groups injected with sesame oil (table 8) the incidence of spontaneous mammary fibroadenoma is 6.7% for 3 groups of 20 rats given AECSC and 15% for those given sugar solution alone. The incidence of spontaneous renal cancer is 1.7% for the groups given AECSC and zero for the groups given sugar solution. Breast cancer occurred in 3.3% of the animals in each group (AECSC versus sugar). When corrections are made for the incidence of spontaneous cancer, B(a)P appears to be a relatively weak carcinogen when the compound is administered by feeding, conclusions reached earlier by Van Duuren, et al. (13). The occurrence of a higher incidence of uterine pathology in the groups given AECSC to drink could be a chance occurrence; otherwise, there is no evidence that AECSC influences the incidence of spontaneous tumors.

Table 8. Pathology observed in all control (injected with or fed sesame oil) Sprague-Dawley strain female rats given aqueous extract of cigarette smoke condensate (AECSC) 0.25 mg/ml 5% sugar solution or sugar solution alone as drinking fluid for 1 yr., age 30 to 400 da.

	AECSC in 5% Sugar Sol.	5% Sugar Sol.
No. rats	60	60
Mammary adenocarcinoma	2	2
Mammary fibroadenoma	4	9
Renal adenocarcinoma	1	0
Rats without tumor	53	49
Cataract	3	1
Milk in mammae	5	3
Uterine pathology:	13	5
Polyps	1	2
Hematometria	4	0
Pyometria	8	3
Ovarian pathology	0	0

DISCUSSION

The consensus of opinion of investigators concerned with carcinogenicity of tobacco smoke is that the assay of individual fractions does not account for the tumorigenicity observed in whole smoke condensate and that the most active fractions are concentrated in the non-aqueous phase. For this reason little attention has been paid to the aqueous extract. This material is an extremely complex mixture, and even under the most carefully standardized conditions the concentration of the components will vary with different batches of the same cigarette; therefore it is not possible to attribute the effects observed to any class of compounds. On the basis of present analytical data, each rat would consume, at best, not more than 60 μg nicotine and 30 μg phenol per ml of AECSC solution. Since the average daily consumption of fluid per rat was 15 ml, it is unlikely that phenols are responsible in view of the large quantity required to produce a minimal co-carcinogenic effect in mice (1).

In a recent report Dontenwill, et al. (34) observed that a fraction of tobacco smoke condensate which contained almost all the aromatic hydrocarbons and a number of water-soluble compounds accounted for most of the tumorigenic activity of cigarette smoke condensate. These investigators concluded that substances other than aromatic hydrocarbons in tobacco smoke condensate were responsible, in part, for the induction of skin tumors in mice. Although Whitehead and Rothwell (5) attached no significance to the observation that their aqueous extract of cigarette smoke condensate induced a low incidence of tumors in mice, it is to be noted that their two fractions, the aqueous and non-aqueous fractions together, accounted for the tumorigenicity of their whole cigarette smoke condensate.

Acceleration of B(a)P-induced sarcoma by AECSC which has no detectable influence on B(a)P-induced breast cancer or spontaneous breast tumors is a paradox. However, the observations presented in this report are in line with those of Bingham and Falk (35), who demonstrated that the effect of carcinogens in mice is most apparent during exposure to low concentrations of B(a)P. Our experiments shed no light on how the effect of AECSC is mediated, whether directly or indirectly via metabolic processes. Nor can we judge the relevance of the observations presented in this report to the smoking and health question, since the method employed for preparing AECSC undoubtedly modifies its composition relative to fresh tobacco smoke. However, they do point up the need for further exploration of the effects produced by the aqueous fraction and the identification of the component(s) involved.

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Table App. 1—Mean (± S.E.) organ and body weights of Sprague-Dawley female rats given aqueous extract of cigarette smoke condensate (AECSC) 0.25 mg/ml 5% sugar solution to drink. AECSC was the sole source of drinking fluid from 30 days of age until sacrifice, 33 ± 3 days following the detection of the first palpable tumor. Animals injected with sesame oil were observed 22 wk. Benzo(a)pyrene, dissolved in 0.1 ml sesame oil, was administered by subcutaneous injection on alternate days for 30 doses, age 30-90 da.

	AECSC			
	Dose of Benzo(a)pyrene (μg)			
	400	200	100	Sesame Oil
Gross Body Weight	241 ± 3.5 gm	244 ± 3.9 gm	261 ± 6.3 gm	229 ± 3.3 gm
Net Body Weight *	230 ± 3.1	233 ± 0.9	230 ± 3.8	229 ± 3.3
Liver	9.24 ± 0.21	9.12 ± 0.27	10.08 ± 0.40	7.04 ± 0.18
Spleen	0.692 ± 0.053	0.680 ± 0.048	0.887 ± 0.081	0.490 ± 0.015
Kidneys **	1.82 ± 0.03	1.88 ± 0.04	1.88 ± 0.03	1.87 ± 0.03
Heart	0.795 ± 0.015	0.774 ± 0.015	0.754 ± 0.014	0.686 ± 0.023
Brain	1.54 ± 0.22	1.50 ± 0.01	1.55 ± 0.02	1.55 ± 0.04
Lungs **	1.41 ± 0.03	1.37 ± 0.03	1.24 ± 0.04	1.11 ± 0.04
Uterus	0.525 ± 0.024	0.506 ± 0.024	0.472 ± 0.027	0.554 ± 0.023
Thymus	0.148 ± 0.007	0.161 ± 0.005	0.133 ± 0.010	0.147 ± 0.002
Adrenals **	0.071 ± 0.003	0.069 ± 0.001	0.069 ± 0.003	0.063 ± 0.002
Ovaries **	0.071 ± 0.003	0.080 ± 0.003	0.085 ± 0.004	0.072 ± 0.004
Pituitary	0.015	0.015	0.014	0.015

* Net body weight = mean gross body weight minus mean total tumor weight per rat
** Paired organs

Table App. 2—Mean (± S.E.) organ and body weights of Sprague-Dawley female rats given 5% sugar solution to drink. The quantity of sugar solution was restricted to the volume consumed by rats drinking aqueous extract of cigarette smoke condensate (AECSC) and was the sole source of drinking fluid from 30 days of age until sacrifice 33 ± 3 days following the detection of the first palpable tumor. Animals injected with sesame oil were observed for 22 wk. Benzo(a)pyrene, dissolved in 0.1 ml sesame oil, was administered by subcutaneous injection on alternate days for 30 doses, age 30-90 days.

	5% Sugar Solution			
	Dose of Benzo(a)pyrene (μg)			
	400	200	100	Sesame Oil
Gross Body Weight	244 ± 6.3 gm	255 ± 9.8 gm	256 ± 7.2 gm	233 ± 2.9 gm
Net Body Weight *	219 ± 5.1	226 ± 3.6	227 ± 4.2	233 ± 2.9
Liver	9.24 ± 0.71	9.30 ± 0.50	9.14 ± 0.46	7.50 ± 0.23
Spleen	0.795 ± 0.109	0.779 ± 0.077	0.847 ± 0.118	0.488 ± 0.017
Kidneys **	1.79 ± 0.08	1.79 ± 0.06	1.83 ± 0.03	1.82 ± 0.04
Heart	0.774 ± 0.010	0.747 ± 0.020	0.740 ± 0.015	0.706 ± 0.017
Brain	1.53 ± 0.02	1.53 ± 0.03	1.50 ± 0.03	1.55 ± 0.04
Lungs **	1.44 ± 0.07	1.35 ± 0.09	1.29 ± 0.04	1.15 ± 0.02
Uterus	0.499 ± 0.035	0.512 ± 0.066	0.523 ± 0.034	0.580 ± 0.024
Thymus	0.168 ± 0.013	0.148 ± 0.010	0.167 ± 0.015	0.142 ± 0.007
Adrenals **	0.067 ± 0.004	0.064 ± 0.003	0.063 ± 0.003	0.058 ± 0.002
Ovaries **	0.082 ± 0.005	0.079 ± 0.003	0.075 ± 0.004	0.077 ± 0.004
Pituitary	0.015	0.013	0.013	0.015

* Net body weight = Mean gross body weight minus mean total tumor weight per rat
** Paired organs

Table App. 3—Mean (± S.E.) body and organ weights of Sprague-Dawley strain female rats given a subcutaneous injection of Benzo(a)pyrene (BP) in 0.1 ml sesame oil or sesame oil alone on alternate days for 30 doses (age 30 to 90 days) and aqueous extract of cigarette smoke condensate (AECSC) 0.25 mg/ml 5% sugar solution as drinking fluid from 30 days of age until autopsy. Tumor-bearing rats were killed 33 ± 3 days after the detection of the first palpable tumor. Control animals were observed for one year.

BP (dose, µg)	50	25	12.5	3	—
Body wt. (gross)	252. ± 7.	256. ± 6.	260. ± 7.	263. ± 5.	255 ± 4.
Body wt. (net)*	232.	231.	235.	255.	---
Tumor wt. (total)	20.12 ± 5.30	25.2 ± 6.7	24.7 ± 6.9	8.4 ± 2.8	
Liver	8.95 ± 0.36	8.70 ± 0.34	8.41 ± 0.24	8.50 ± 0.28	7.54 ± 0.22
Spleen	0.78 ± 0.08	0.75 ± 0.09	0.63 ± 0.06	0.63 ± 0.05	0.53 ± 0.01
Kidneys **	2.01 ± 0.05	1.92 ± 0.04	2.01 ± 0.03	2.24 ± 0.07	2.21 ± 0.03
Heart	0.77 ± 0.01	0.74 ± 0.01	0.75 ± 0.01	0.84 ± 0.03	0.87 ± 0.01
Brain	1.64 ± 0.01	1.62 ± 0.01	1.68 ± 0.01	1.70 ± 0.02	1.64 ± 0.01
Lungs **	1.34 ± 0.04	1.30 ± 0.04	1.24 ± 0.14	1.43 ± 0.13	1.34 ± 0.02
Uterus	0.50 ± 0.03	0.52 ± 0.03	0.45 ± 0.02	0.71 ± 0.06	0.78 ± 0.03
Thymus	0.149 ± 0.01	0.163 ± 0.001	0.142 ± 0.006	0.126 ± 0.008	0.073± 0.004
Adrenals **	0.063 ± 0.002	0.059 ± 0.005	0.058 ± 0.001	0.059 ± 0.001	0.057± 0.001
Ovaries **	0.083 ± 0.006	0.092 ± 0.005	0.095 ± 0.005	0.100 ± 0.004	0.091± 0.004
Pituitary	0.012	0.011	0.013	0.013	0.015

* Net body weight = gross body weight minus total tumor weight
** Paired organs

Table App. 4—Mean (± S.E.) body and organ weights of Sprague-Dawley strain female rats given a subcutaneous injection of Benzo(a)pyrene (BP) in 0.1 ml sesame oil or sesame oil alone on alternate days for 30 doses (age 30 to 90 days) and 5% sugar solution as drinking fluid from 30 days of age until autopsy. The quantity of sugar solution was restricted to the volume consumed by rats drinking aqueous extract of cigarette smoke condensate (AECSC) in 5% sugar solution. Non-tumor bearing rats were observed for one year.

BP (dose, μg)	50	25	12.5	3	—
Body wt. (gross)	245. ± 4.	248. ± 4.	251. ± 5.	259. ± 3.	271. ± 7.
Body wt. (net)*	234.	232.	234.	255.	---
Tumor wt. (total)	11.5 ± 2.2	16.5 ± 3.6	17.3 ± 4.4	4.8 ± 1.7	
Liver	8.59 ± 0.26	9.03 ± 0.25	8.78 ± 0.41	8.01 ± 0.24	7.19 ± 0.22
Spleen	0.63 ± 0.04	0.72 ± 0.06	0.64 ± 0.06	0.52 ± 0.02	0.50 ± 0.01
Kidneys **	2.02 ± 0.04	1.95 ± 0.05	1.98 ± 0.05	2.16 ± 0.05	2.20 ± 0.05
Heart	0.75 ± 0.01	0.74 ± 0.01	0.77 ± 0.02	0.81 ± 0.01	0.91 ± 0.02
Brain	1.65 ± 0.02	1.69 ± 0.09	1.64 ± 0.02	1.68 ± 0.01	1.69 ± 0.02
Lungs **	1.26 ± 0.07	1.29 ± 0.03	1.27 ± 0.03	1.31 ± 0.10	1.33 ± 0.03
Uterus	0.50 ± 0.03	0.46 ± 0.03	0.53 ± 0.02	0.77 ± 0.05	0.71 ± 0.04
Thymus	0.174 ± 0.011	0.151 ± 0.001	0.152 ± 0.006	0.099 ± 0.009	0.078 ± 0.005
Adrenals **	0.060 ± 0.001	0.058 ± 0.001	0.061 ± 0.002	0.058 ± 0.001	0.055 ± 0.001
Ovaries **	0.087 ± 0.005	0.088 ± 0.005	0.087 ± 0.004	0.093 ± 0.005	0.087 ± 0.004
Pituitary	0.013	0.013	0.013	0.015	0.016

* Net body weight = gross body weight minus total tumor weight
** Paired organs

Table App. 5—Mean body and organ weights of Sprague-Dawley female rats given aqueous extract of cigarette smoke condensate (AECSC) 0.25 mg/ml in 5% sugar solution or 5% sugar solution alone as drinking fluid for one year beginning at age 30 days. All rats were fed a single 100-mg dose of 3,4-Benzpyrene (BP) in 5 ml sesame oil or sesame oil alone by gastric tube at age 50 days.

Drinking Fluid	AECSC		Sugar Solution	
Treatment	BP	Sesame Oil	BP	Sesame Oil
No. rats	17	19	16	19
Body weight	261.	261.	260.	251.
Liver	8.146	7.65	8.087†	7.152
Spleen	0.674	0.513	0.506	0.502
Kidneys *	2.165	2.156	2.217	2.074
Heart	0.866	0.847	0.793	0.826
Brain	1.688	1.681	1.667	1.672
Lungs *	1.390	1.327	1.278	1.272
Uterus	0.984	0.828	0.935	0.865
Thymus	0.086	0.077	0.080	0.077
Adrenals *	0.058	0.058	0.056	0.054
Ovaries *	0.107	0.092	0.094	0.093
Pituitary	0.0151	0.0143	0.0145	0.0176

† Weight significantly different from control, P = 0.01
* Combined weight of paired organs

Table App.6—Mean body and organ weights of Sprague-Dawley female rats given aqueous extract of cigarette smoke condensate (AECSC) 0.25 mg/ml in 5% sugar solution or 5% sugar solution alone for one year beginning at age 30 da. All rats were fed 3,4-Benzpyrene, B(a)P, 20 mg in 1 ml sesame oil or 1 ml sesame oil by gastric tube at weekly intervals for 5 doses beginning at age 50 da.

Drinking Fluid	AECSC		Sugar Solution	
Treatment	B(a)P	Sesame oil	B(a)P	Sesame oil
No. rats	16	18	15	18
Body weight	267.	263.	259.	259.
Liver	7.970†	7.525	7.842	7.249
Spleen	0.603	0.525	0.539	0.507
Kidneys*	2.159	2.074	2.185	2.097
Heart	0.904	0.895	0.847	0.860
Brain	1.728	1.656	1.683	1.664
Lungs*	1.389	1.302	1.324	1.313
Uterus	0.906	0.790	0.685	0.780
Thymus	0.088	0.078	0.085	0.077
Adrenals*	0.064	0.057	0.061	0.057
Ovaries*	0.097	0.092	0.097	0.096
Pituitary	0.0144	0.0130	0.0157	0.0128

†Significantly different from control, P = .05
*Combined weight of paired organs

Text-figure 1. Method of preparation of aqueous extract of cigarette smoke condensate (AECSC).

Preparation of Water-Soluble Extract of Cigarette Smoke Condensate*

Step 1 - Smoke increments of 2 M cigarettes.

2 - Collect in glass trap cooled with CO_2-acetone refrigerant.

3 - Dissolve condensate in acetone-water (80:20), 0.5 ml/cigarette, with ultrasonic dispersion for 10 sec.

4 - Divide sample into two parts: remove solvent from each part separately in rotary evaporator, 20" Hg at 35°. Wash with acetone-water (80:20).

5 - Combine condensates and washings.

6 - Remove acetone under reduced pressure (28" Hg).

7 - Add 200 ml distilled water.

8 - Transfer to separatory funnel and extract the lower aqueous phase.

9 - Wash upper (insoluble) phase with 50-100 ml distilled water and add wash to aqueous phase.

10 - Allow aqueous extract to settle 48 hrs. at 4°.

11 - Determine total solids per ml, viz:

(1) Pipette 1-10 ml aqueous extract tobacco smoke condensate into tared weighing containers. Make triplicate determinations.

(2) Dry under nitrogen (25" Hg, 50°).

(3) Weigh samples at 5-minute intervals until constant weight is attained.

12 - Dilute to final concentration of 20 mg solids/ml with distilled water.

13 - Repeat total solids determination on diluted extract.

14 - Store in actinic glassware.

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* Prepared by Dr. John Green, Department of Agronomy, University of Ky.

Text-figure 2. Influence of an aqueous extract of cigarette smoke condensate (AECSC) on mean body weight of Sprague-Dawley female rats. AECSC, dissolved in 10% sugar solution, was administered as drinking fluid to 4 groups of 6 rats for 10 weeks, age 30 to 100 days. Control groups drank the same quantity of 10% sugar solution consumed by the experimental group drinking AECSC. Concentration refers to mg/ml 10% sugar solution. At point 1 (arrow) all groups were given 200 ml 10% sugar solution; at point 2, AECSC was first given to the experimental groups; at point 3 fluid was appropriately restricted in the control groups. An estimate of AECSC consumed by each rat/day was: 1.0 mg/ml, 6.7 mg, 0.5 mg/ml, 6.0 mg, 0.25 mg/ml, 4.2 mg, and 0.125 mg/ml, 3.8 mg respectively.

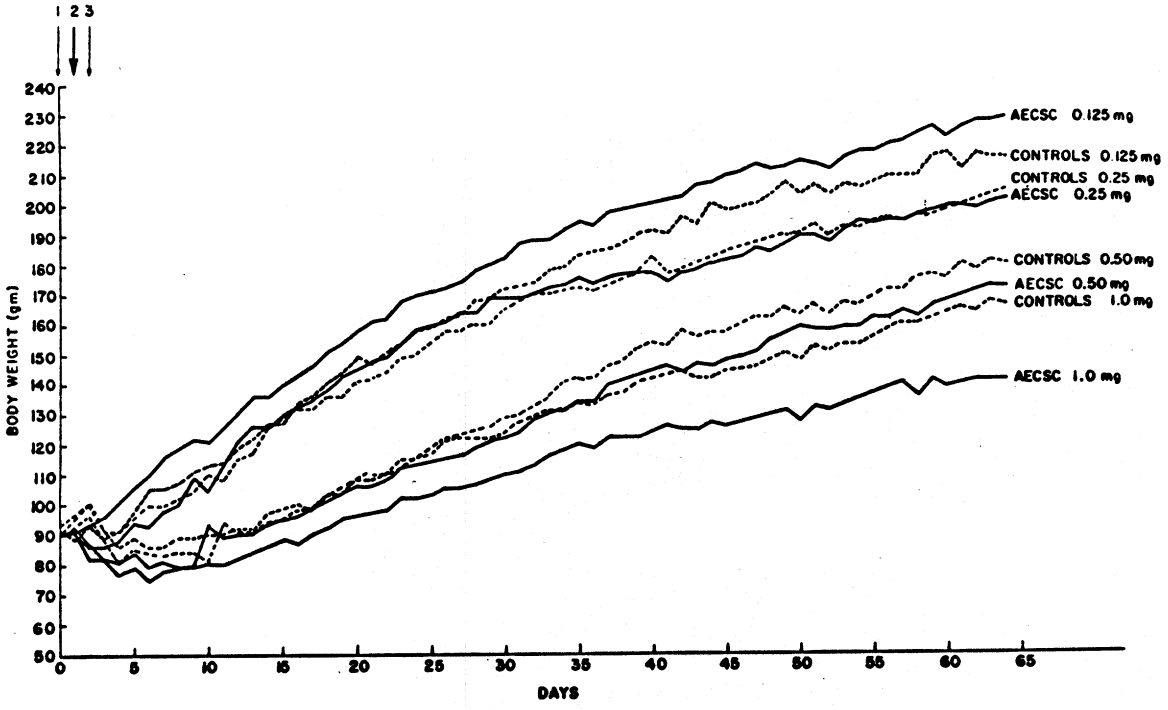
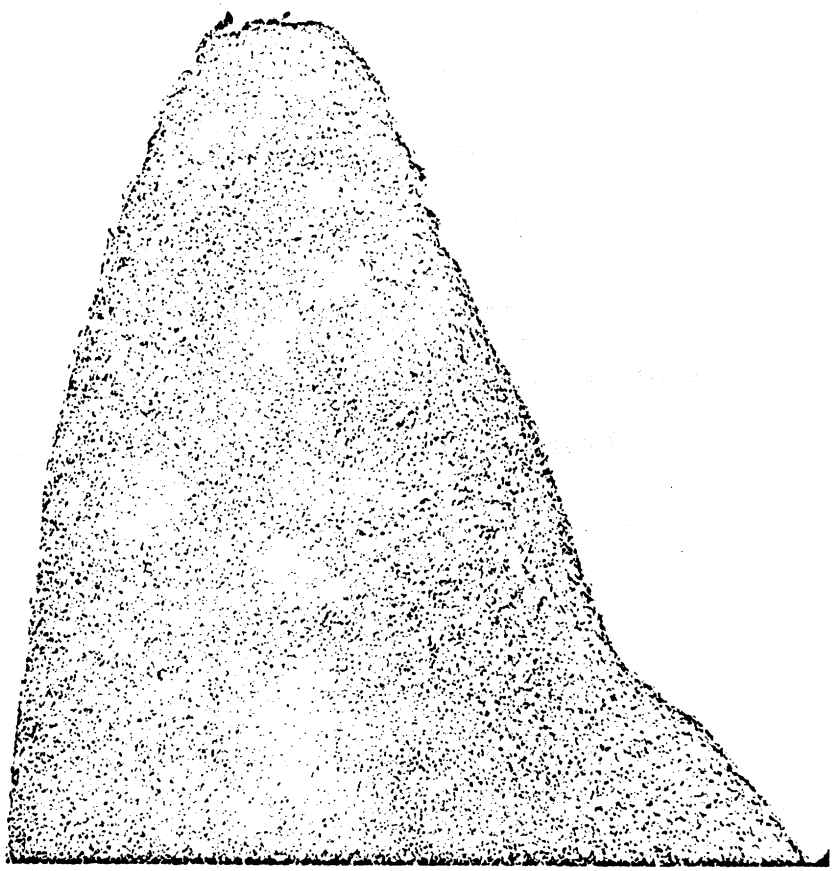

1 2 3
BODY WEIGHT (gm)
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
0
5
10
15
20
25
30
35
40
45
50
55
60
65
DAYS
AECSC 0.125 mg
CONTROLS 0.125 mg
CONTROLS 0.25 mg
AECSC 0.25 mg
CONTROLS 0.50 mg
AECSC 0.50 mg
CONTROLS 1.0 mg
AECSC 1.0 mg

Figure 1-A. Spleen of control rat age 14 months. X 234.

Figure 1-B. High power magnification of spleen shown in Figure 1-A. X 910.



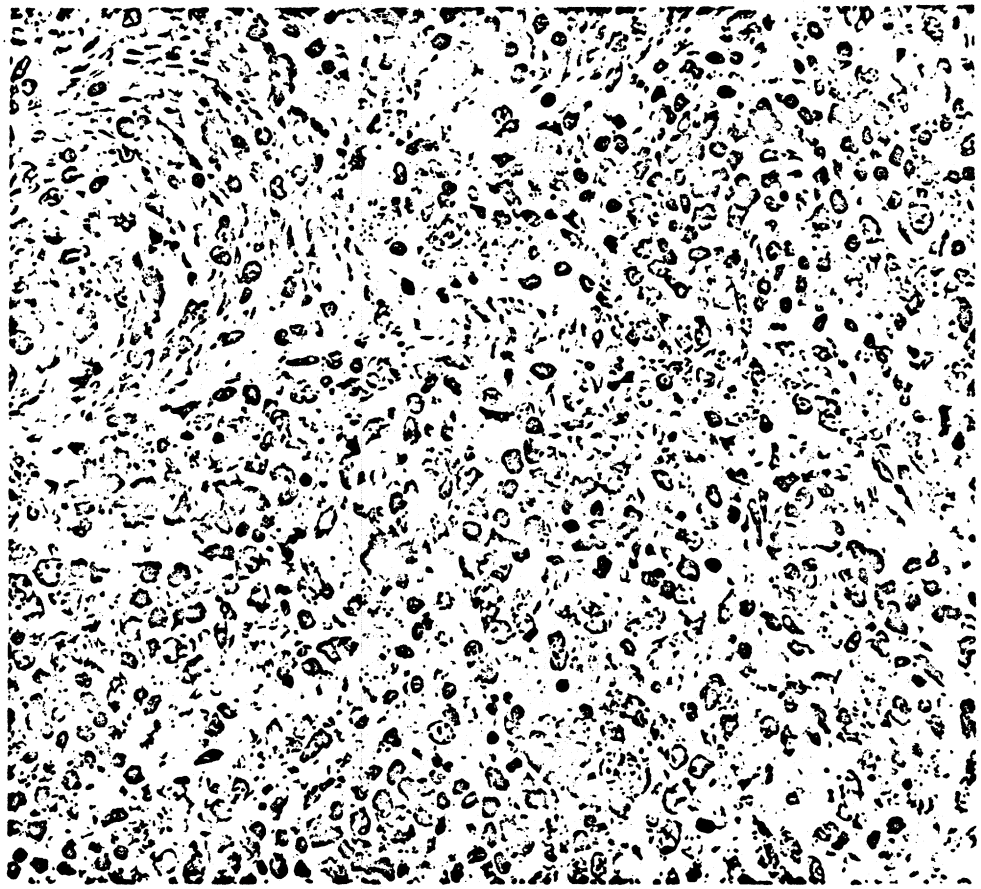
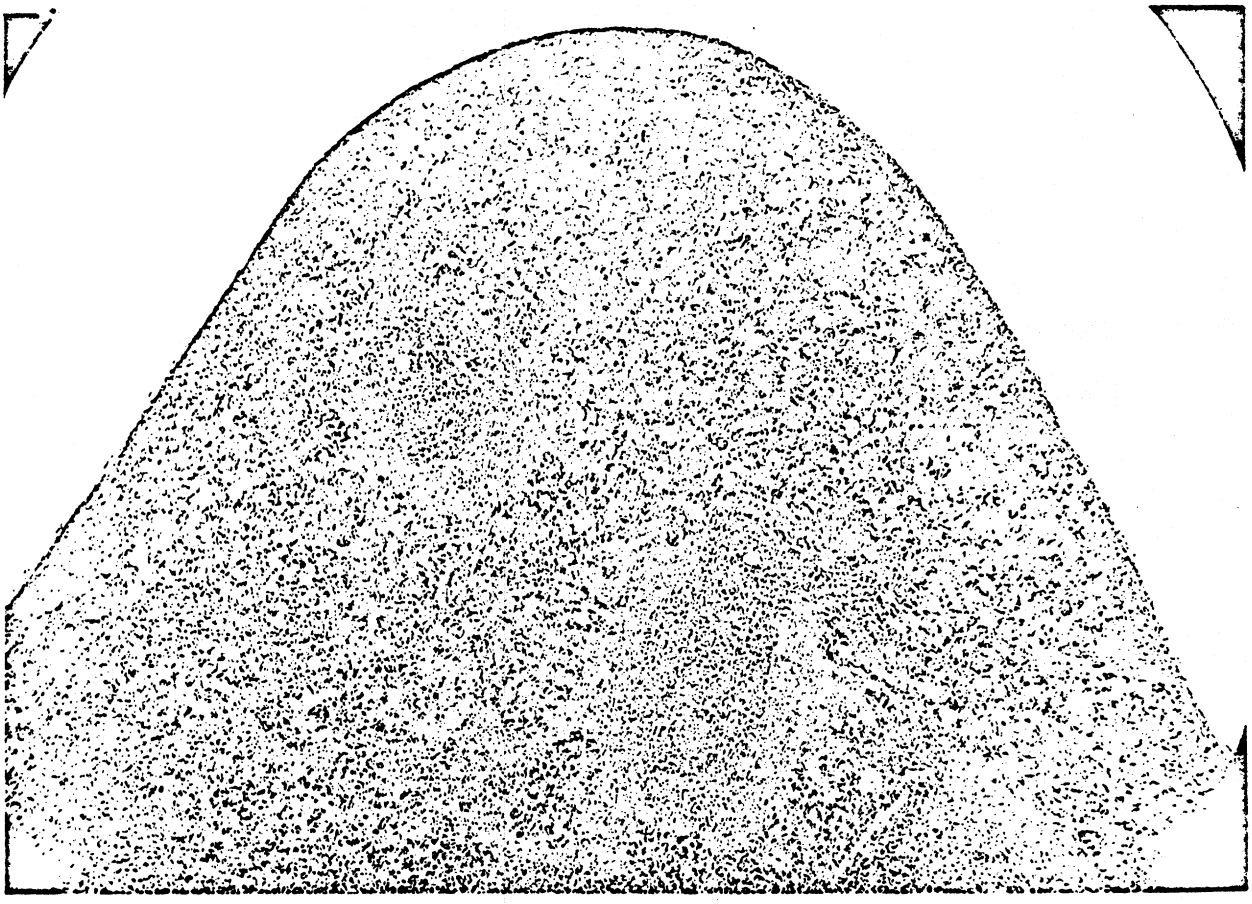


Figure 2-A. Spleen of rat bearing subcutaneous sarcoma weighing 63 gm showing myeloid hyperplasia. X 234.

Figure 2-B. High power magnification of spleen shown in Figure 2-A. X 910.



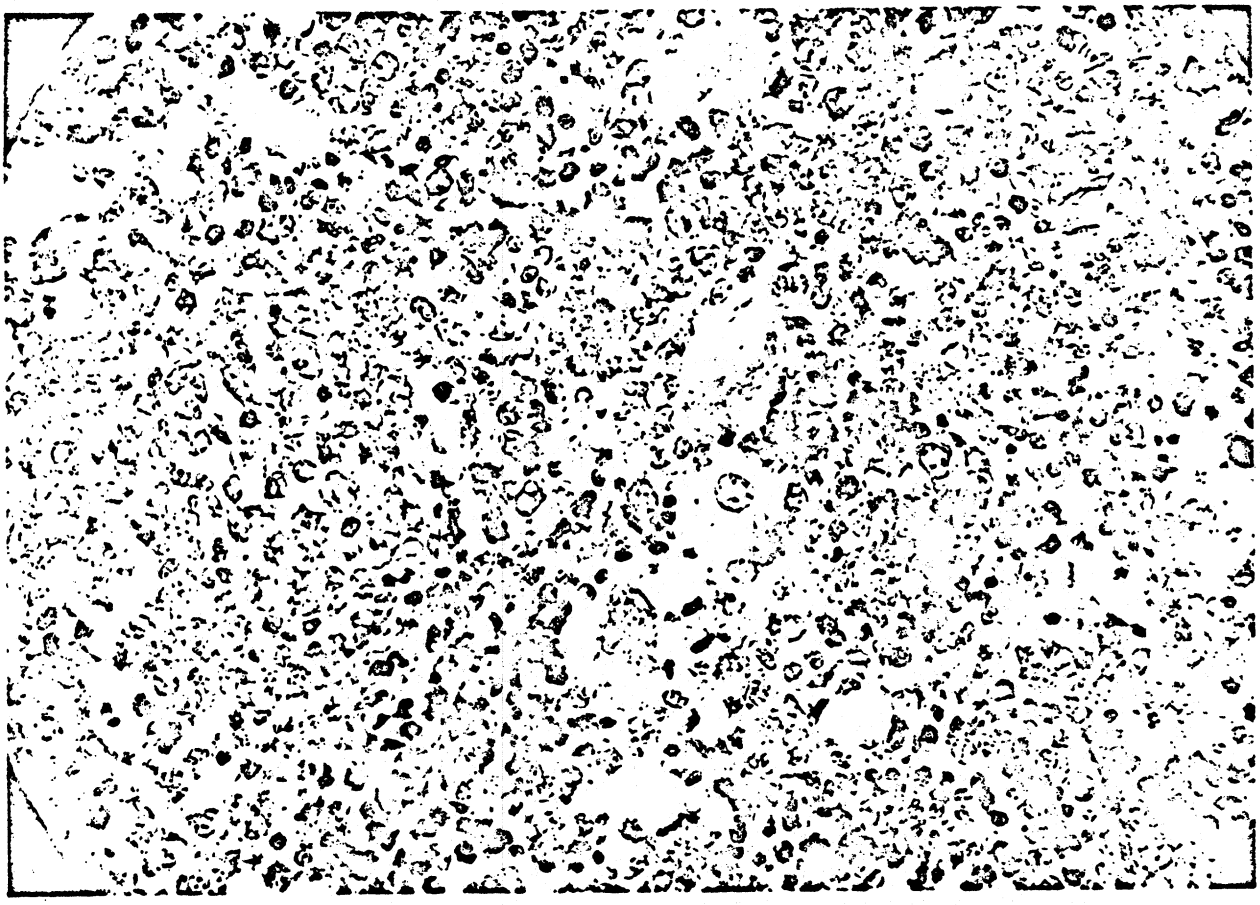


Figure 3. Lymphocytoid cells in peripheral blood of tumor-bearing rat. X 2310.

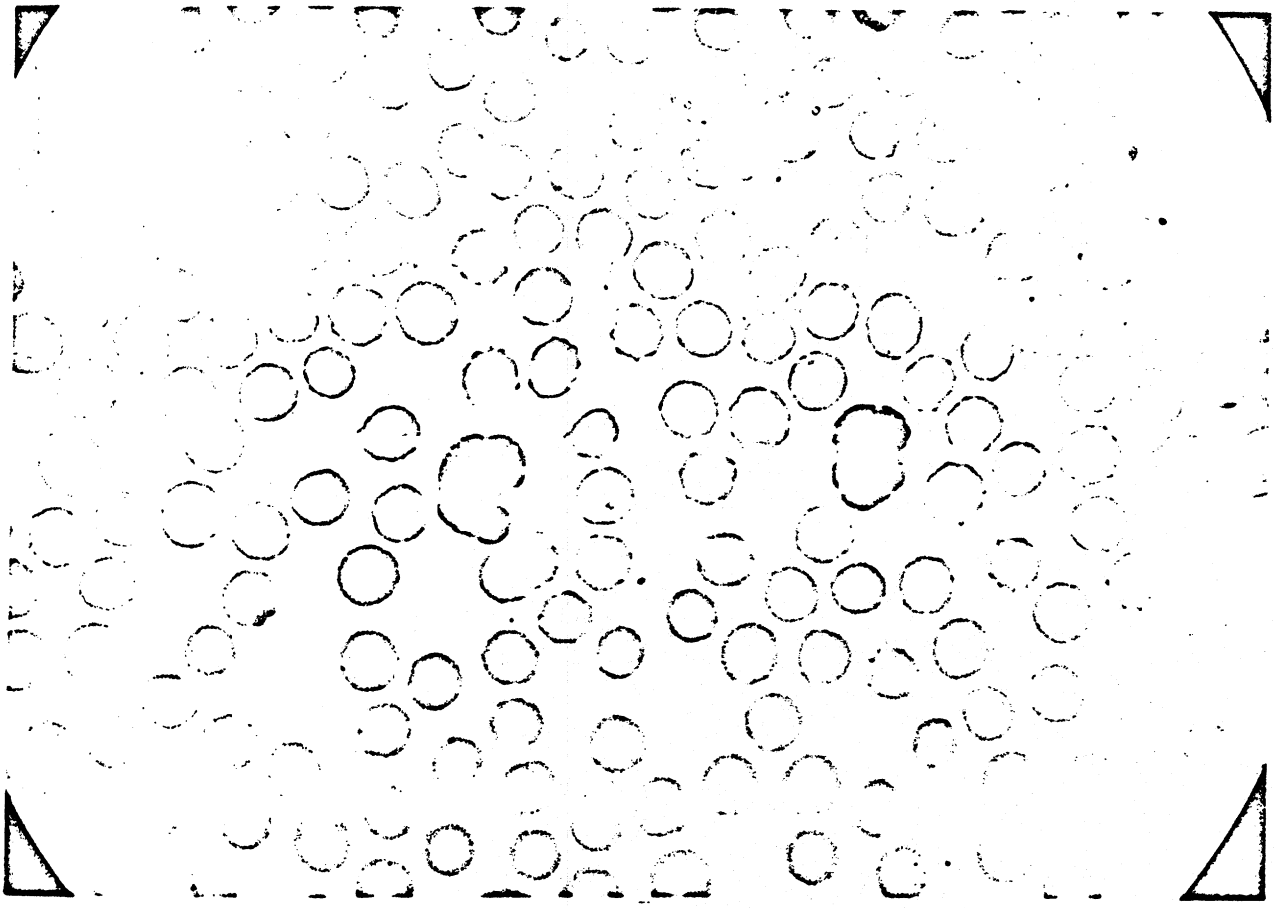


Figure 4-A. Adenocarcinoma of kidney. X 234.

Figure 4-B. High power magnification of kidney shown in Figure 4-A. X 910.